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轉基因策略發展雙生病毒抗性植物之現況及展望

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摘　要

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雙生病毒 (geminivirus) 為全球性的重要植物性病毒病原，可危害多種重要經濟及糧食作物，造成重大之經濟損失，嚴重影響農民收入與糧食供應。因此發展有效防治此病毒病害的策略有其重要性及應用價值。由於此病毒之基因體結構由單股 DNA (ssDNA) 組成，且易發生病毒之間的重組而具高度變異性，因此一般使用之傳統防治方法效果不盡理想且不易獲得高度抗性之植物。因此利用遺傳工程技術為理論基礎，發展出有效的轉基因抗性，提供了另一有效的病毒防治途徑。本文針對目前利用轉基因策略發展雙生病毒抗性的相關研究作完整之彙整，包括藉由表現病毒蛋白、非病毒蛋白、基因沉寂與 DNA 干擾等策略來促使轉基因植物表現抗性等，以期能了解有效率的轉基因抗病模式，作為研發持久且具廣泛性抗性轉基因植物的基石。

關鍵詞：雙生病毒、轉基因植物、病毒抗性

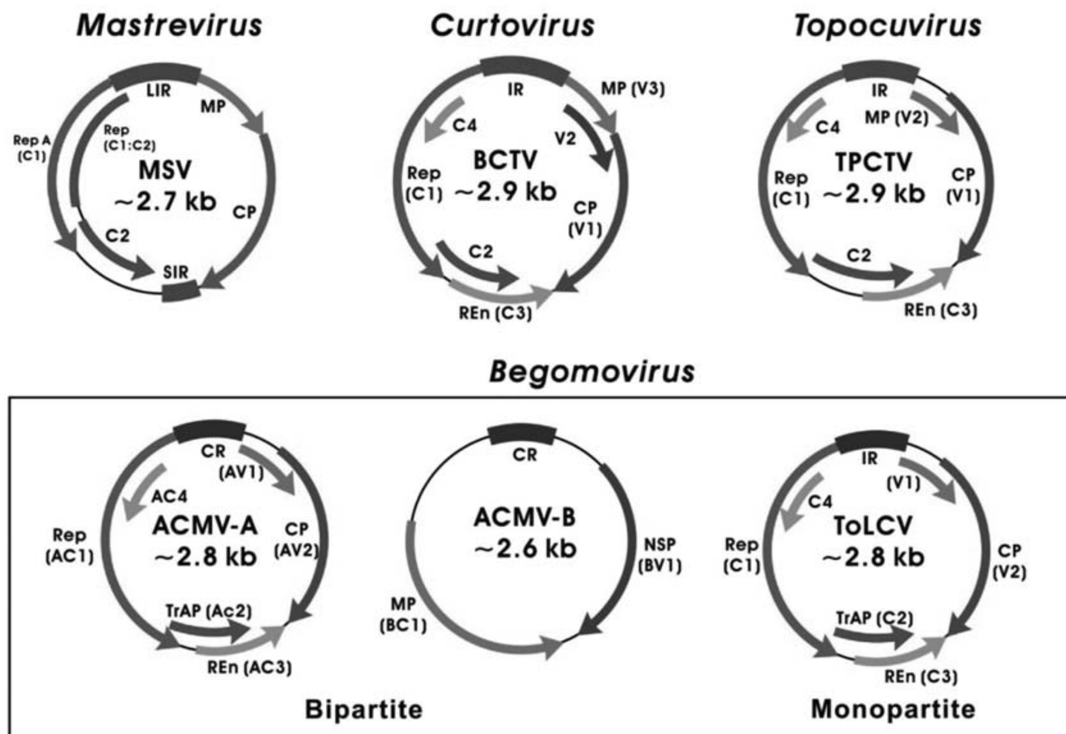
緒　言

雙生病毒 (geminiviruses) 已知可危害世界上許多高經濟價值及糧食作物，包括棉花、樹薯、甘藷、大豆、菸草、甜菜、萵苣、番茄及瓜類等。病害發生嚴重時可完全摧毀作物的生產，造成農業產量及經濟上重大的損失，尤其是熱帶及亞熱帶地區更為嚴重 [113]。由於此病毒的基因體具有高度重組性，致使病毒歧異度增加，越來越多新種的雙生病毒持續地被發現；再者病毒的媒介昆蟲族群密度提高及殺蟲劑的不當施用，相對增加了病媒抗藥性產生的可能性；加上全球暖化的影響，更促使病害的發生由熱帶、亞熱帶地區逐漸擴展至溫帶地區 [90]，對重要的經濟作物造成越來越大的威脅，儼然已成全球性的重要植物病毒病原。

雙生病毒是由兩個不完整之球形組成的雙生 (gemini) 病毒顆粒，顆粒內具有環狀單股基因體 DNA (circular single-stranded DNA, circular ssDNA)。其病毒分類地位屬於雙生病毒科 (*Geminiviridae*)，依基因體組成、傳播媒介及寄主範圍之不同可分為四屬：*Mastrevirus*、*Curtovirus*、*Topocuvirus* 及 *Begomovirus* (圖一) [99]。*Mastrevirus* 的基因體組成為此病毒科中較為獨特的一群 (圖一)，其寄主局限於單子葉禾本科植物，唯一例外是 *Tobacco yellow dwarf virus* 能感染雙子葉植物。*Curtovirus* 之寄主植物含 44 科 300 餘種雙子葉植物。上述二屬病毒之基因體皆為單一環狀單股 DNA (單基因體，monopartite)，以葉蟬 (leafhopper) 類為媒介昆蟲 [99]。*Topocuvirus* 目前只有一個成員即 *Tomato pseudo curly top virus*，能感染雙子葉植物，其基因體為單基因體，媒介昆蟲為樹蟬類 (treehopper) [113]。*Begomovirus* 屬病毒之寄主限雙子葉植物，基因體為一或二個 (雙基因體，bipartite) 環狀單股 DNA，以

粉蝨 (*Bemisia* spp.) 爲媒介昆蟲[99]。超過 80% 以上已知之雙生病毒皆屬於 *Begomovirus*，且多數都爲雙基因體[31]。因此和雙生病毒相關的重大病害中大多數都是由 begomoviruses 所引起，而這類病毒引起的典型病徵爲葉緣捲曲、葉片黃化、植株矮化、及開花減少等現象。

以 *Begomovirus* 屬爲例，病毒基因體由兩個環狀單股 DNA 基因體組成，大小約 2.5-3.0 kb，分別定義爲 DNA-A 及 DNA-B；僅少數病毒由一個環狀單股 DNA 基因體組成，其構造功能皆與 DNA-A 十分相似。DNA-A 基因體包含六個開放讀碼區 (open reading frame, ORF)，其中 2 個位於病毒的正股 (virion sense)，另外 4 個在病毒的互補股 (complementary sense)。DNA-B 上有 2 個開放讀碼區，在病毒的正股及互補股上各有一個 (圖一)[99]。各基因體DNA除了開放讀碼區域外，尚有一個似髮夾狀結構的區域 (stem-loop)，此結構位於基因間區域 (intergenic region, IR)[99]，在 loop 中包含有 *Geminiviridae* 特有的保留序列 (conserved sequence)：TAATATTAC。IR 亦具有雙方向啓動子 (bidirectional promoter) 的特性，可用於轉錄正股及互補股上的基因[46]。此外此屬病毒可能伴隨衛星 DNA (satellite DNA) 的存在，稱之爲 DNA-β，大小約 1.3-1.4 kb，其上具有一個開放讀碼區是爲 βC1，研究報告指出 βC1 與病徵表現相關[25]，同時在某些病毒內亦扮演基因沉寂作用抑制子的角色[24, 41, 57]。目前已於數種單基因體病毒中發現衛星 DNA 的存在，包括 *Ageratum yellow vein virus* (AYVV)[56, 89]、*Cotton leaf curl Multan virus* (CLCuMV)[14]、*Bhendi yellow vein mosaic virus* (BYVMV)[55]、*Malvastrum yellow vein virus* (MYVV)[44] 及部分引起番茄黃化捲葉病 (tomato yellow



圖一、雙生病毒屬之基因體組成。圓弧狀的箭頭代表病毒互補股 (C) 與病毒正股 (V) 的開放讀碼區，病毒互補股與病毒正股之間以基因間隔區 (intergenic region) 作爲間隔。開放讀碼區所轉譯出的蛋白包括: 鞘蛋白 (CP)、移動蛋白 (MP)、病毒基因轉錄促進子 (TrAP)、病毒複製時的促進因子 (REn)、核穿梭蛋白 (NSP) 與病毒複製相關蛋白 (Rep)。

Fig. 1. Genome organization of representative members of the four geminivirus genera. The typical virus of each genus is as the model. MSV, *Maize streak virus*; BCTV, *Beet curly top virus*; TPCTV, *Tomato pseudo-curly top virus*; ACMV, *African cassava mosaic virus*; ToLCV, *Tomato leaf curl virus*. Curved arrows indicate open reading frames (ORFs), including complementary (C) and virion (V) senses and they diverge from an intergenic region (IR) in curtoviruses and topocuviruses, along intergenic region (LIR) and short intergenic region (SIR) in mastreviruses, and a common region (CR) in bipartite begomoviruses. CP, coat protein; MP, movement protein; TrAP, transcription activator protein; REn, replication enhancer protein; NSP, nuclear shuttle protein; Rep, replication-associated protein.

leaf curl disease, TYLCD) 的相關病毒[25, 56, 67]。*Begomovirus* 屬病毒複製是以滾動環狀複製 (rolling circle replication) 方式於寄主細胞核中進行[46]。其過程先以病毒正股 ssDNA 爲模板合成互補股，形成雙股 (dsDNA)，是爲複製型 DNA (replicative form; RF)，接著病毒複製相關蛋白 (replication-associated protein; Rep)，再於正股之 IR 區域的髮夾狀結構中的特定位置 (保留序列 TAATATT↓AC 的第 7 與第 8 個核酸) 形成缺口[46]，促使病毒開始複製合成出正股，最後則是將合成出之線狀 ssDNA 黏合成環狀，完成病毒複製過程[62]。此外，此屬病毒只能經由粉蝨傳播，其傳毒模式屬循環型 (circulative mode) 傳播，病毒在蟲體具繁殖現象 (multiplication)，並永續性的存留在粉蝨，且一經獲毒後，終身帶毒，甚至可傳留給子代[38]。寄主植物感染病毒後會造成葉片黃化及捲曲等病徵，造成的經濟損失可高達100%。

雙生病毒之防治策略

由於雙生病毒具高度農業經濟重要性，尤其以易因基因體重組而導致病毒具高歧異度之 begomoviruses 更爲重要，因此不少研究致力於發展有效防治此病毒感染的策略，一般使用的策略包括：(1) 抗病育種－由於雙生病毒具有高度的變異性，欲篩選出合適的抗病基因來源且獲得有效抗病品種有一定的困難點[60]，以番茄爲例，在過去幾十年裡所篩選獲得之抗病品種，其園藝性狀往往不佳，如產量低等等，且會因地域或病毒分離株 (isolate) 不同，其抗病程度亦會有所不同；耐病品種雖有利於農業生產，但受病毒感染後即成爲新的感染源，嚴重威脅感病品種及其他寄主作物[40, 61]。(2) 管理昆蟲傳播媒介－使用 50 孔目 (mesh) 圍網阻止粉蝨侵入是目前效果較佳的防治方法[48]，但其花費成本高，農民不易接受。若是利用殺蟲劑來達到減少蟲媒的目的，除了高成本外，常因媒介昆蟲產生抗藥性導致防治效果不顯著[104]。(3) 農業栽培法 (culture practice)－利用和非寄主植物進行間作 (intercropping)、移除田間殘株或種植圍籬作物如高粱、玉米等方式以期隔離蟲媒及減少病源，然此法需配合施用殺蟲劑或栽種抗/耐病品種，防治效果才較明顯[34, 48]。(4) 轉基因抗性 (transgenic resistance)－近年來由於基因工程技術的突飛猛進，因此現今已可利用基因轉殖技術於植物內表現外來基因以提供植物抗性。其中最廣泛被使用的策略爲利用病原誘導抗病性 (pathogen-derived resistance, PDR) 理論[87]，藉由生產轉基因植物的方式針對病毒繁殖過程中的複製 (replication)、包被 (encapsidation) 及移動 (movement) 進行抑制或干擾來對抗病毒的感染。本文即是收集轉基因策略發展抗雙生病毒轉基因植物之相關研究，依不同機制作分類介紹與彙整。

發展抗雙生病毒植物之轉基因策略

雙生病毒植物之轉基因策略包括病原誘導抗性，主要可分爲蛋白媒介抗性 (protein-mediated resistance) 及RNA媒介抗性 (RNA-mediated resistance) 兩大機制。另外亦有利用複製過程中產生的干擾缺失性 DNA (defective interfering DNA, DI DNA) 發展轉基因抗性者。除了病原誘導抗性外，亦可利用其他非病毒蛋白來產生抗性。

1. 蛋白媒介抗性－表現病毒蛋白

此策略之機制爲病原誘導抗性中的蛋白媒介抗性，亦即在轉基因植物內表現大量的病毒蛋白，藉以干擾病毒在寄主細胞內正常的複製、脫鞘、移動等功能而產生抗性[43]。此理論首先被 Powell-Abel 等人證實，將菸草嵌紋病毒 (*Tobacco mosaic virus*, TMV) 之鞘蛋白導入菸草中可延緩 TMV 之感染[75]。亦有研究指出在具抗性之轉基因植物內可測到大量的轉基因產物累積且此類抗性往往可提供較廣泛的抗性以抵抗親緣關係較遠的病毒[23]。

雙生病毒基因體所表現的蛋白中，病毒複製相關蛋白 (Rep) 爲一多功能的蛋白對於病毒的複製過程與調控病毒基因轉錄上扮演著舉足輕重的角色[10, 28, 33, 62]，此外亦可與寄主植物的蛋白 (如 retinoblastoma-related protein, RBR protein) 或病毒本身的病毒複製促進因子 (replication enhancement; REn) 蛋白相互結合作用，進而促使寄主植物提供利於病毒複製的環境[47, 92]。因此雙生病毒的病毒複製相關蛋白是一個很好的攻擊目標，而由 Rep 所誘發之抗性相關研究較多、效果也較其他病毒蛋白顯著 (表一)。例如在轉基因植物中表現缺失性病毒複製相關蛋白 (truncated protein of Rep, T-Rep) 可提供植物對此病毒的抗性[51, 70, 88, 94, 95]；然而 Rep 所誘發之抗性多具有病毒專一性，即只能抵抗和轉基因同一來源的病毒，而其他具同源性的病毒則無效[3, 17, 18, 70]。例如表現 *Tomato yellow leaf curl Sardinia virus* (TYLCSV) Rep 蛋白的轉基因植物對 TYLCSV 可提供抗性，但與其 Rep 胺基酸相似度分別高達 93% 及 77% 的同源病毒 TYLCSV-ES 與 *Tomato leaf curl virus* (ToLCV-Au) 卻無法提供抗性[17, 18]。也有例子發現可同時抗自己本身以外且親源性較遠的病毒[19, 66]。如表現 *Tomato leaf curl New Delhi virus* (ToLCNDV) Rep 蛋白

表一、以表現病毒蛋白及非病毒物質提供抗性之轉基因策略

Table 1. Transgenic resistance strategies by expression viral protein and non-viral agents

Protein	Virus species	Transgenic plant	References
CP	TYLCV	Tomato	59
CP	BGMV	Beans	6
CP	ACMV	*N. benthamiana*	36
Modified CP	TMoV	*N. tabacum*	97
T-Rep (1-57 a.a.)	ACMV	Protoplasts	50
	ACMV	*N. benthamiana*	51
T-Rep (1-210 a.a.)	TYLCSV	*N. benthamiana*	70, 17
		Tomato	18, 66
Rep gene (AC1)	ACMV	*N. benthamiana*	88
N-Rep (1-160 a.a.)	TYLCNDV	*N. benthamiana*	19
T-Rep (1-129a.a.)	TYLCV-Is [Mild]	Tomato	3
Full length and truncated Rep	MYMV-Vig	*N. tabacum*	96
Mutant Rep	MSV	*Digitaria sanguinalis*	94
T-Rep (1-219 a.a.) mutant	MSV	Maize	95
AC2 (TrAP)	TGMV or BCTV	*N. benthamiana*	102
AL2	TGMV	*N. benthamiana*	45
C4	ToLCV	*N. tabacum* and tomato	58
AC4	ACMV	*Arabidopsis*	22
MP (BR1 or BL1)	TGMV	*N. benthamiana*	115
Mutated version MP	TMoV	*N. tabacum*	30
BV1 or BC1	BDMV	Tomato	55
Non-viral agent			
Dianthin	ACMV	*N. benthamiana*	52
AZPs (artificial zinc finger proteins)	BSCTV	*Arabidopsis*	93
	TYLCV	Tomato	103
Whitefly GroEL	TYLCV	Tomato	2
scFv-ScRep	TYLCV	*N. benthamiana*	85

的轉基因植物可同時抗 *African cassava mosaic virus* (ACMV) (Rep 胺基酸相似度 72%)、*Pepper huasteco yellow vein virus* (PHYVV; Rep 胺基酸相似度 65%) 及 *Potato yellow mosaic virus* (PYMV-TT; Rep 胺基酸相似度 64%) [19]。造成此現象的原因可能是轉基因植物對同源性病毒的抗性在一段時間內會被病毒經由基因沉寂機制抑制轉基因蛋白表現而擊潰，相對的，和轉基因同源性較低的病毒則無法藉此方式而有效抑制轉基因的表現，因此由轉基因蛋白所提供之抗性仍存在 [66]。此外，由於 Rep 蛋白可與寄主植物的蛋白產生相互作用，故於植物內表現全長度 Rep 蛋白時可能影響轉基因植物的正常生長，此問題可藉由表現缺失性蛋白或突變蛋白來加以避免。

雙生病毒的鞘蛋白 (coat protein; CP) 除了組成病毒顆粒之外，亦有報告指出也參與病毒 ssDNA 在寄主細胞內的轉運，可將 ssDNA 由細胞質帶到細胞核，再將新合成的病毒 ssDNA 由細胞核帶到細胞質 [84]。早期研究大多採用鞘蛋白來發展對抗雙生病毒之轉基因作物，例如轉殖單基因體的 TYLCV 之鞘蛋白至番茄中可達到保護效果，但接種病毒後 4 個月內仍可偵測病毒的存在 [59]，然而表現雙基因體病毒的鞘蛋白則效果不彰 [6, 36]。因爲於單基因體病毒中鞘蛋白爲病毒系統性感染所需的蛋白 [16, 84]，而在雙基因體病毒鞘蛋白則非必需蛋白，反而可藉由 DNA-B 中的核穿梭蛋白 (nuclear shuttle protein, NSP) 完成病毒系統性感染 [54, 74]。此外鞘蛋白亦被報導參與媒介昆蟲傳播病毒的機制 [49, 68, 71]，若病毒的鞘蛋白產生缺失則會影響病毒與媒介昆蟲之間的相互作用而減低或喪失傳播病毒的能力，透過此一途徑或許可大幅減少田間的病毒傳播機率並降低病毒接種源。

DNA-B 含有二個開放讀碼區：BV1 和 BC1，分別產生核穿梭蛋白 (NSP) 及移動蛋白 (movement protein, MP)，其中 NSP 具有將病毒 DNA 穿梭植物細胞核膜之功能 [86]；而MP則是使病毒利於在細胞間移動 (cell-to-cell movement) [72]。因兩者主要掌管病毒的移動，故若在植物中表現病毒的移動蛋白則可達到延遲發病的效果 [30, 53, 115]，而少數例子可提供廣泛性的抗性 [30]。然而某些雙基因體的 begomoviruses 中，其 NSP 亦具有致病

性決定因子 (pathogenicity determinants) 的功能，若過度表現 (over-expressed) 則往往會導致負面的效果，如出現不正常的植物表型[53]。故使用時須注意利用有缺失性蛋白或突變的方式進行轉殖。

此外，若將 C4 (或 AC4) 蛋白轉殖到寄主植物中進行表現，則植物的外表型態會出現類似病毒感染的病徵[22, 58]；而轉殖 *Tomato golden mosaic virus* (TGMV) 之病毒基因轉錄促進子蛋白 (transcriptional activator protein; TrAP) 到寄主植物中表現，反而會增加寄主對病毒的感受性 (susceptibility)[45, 102]。這可能是此兩種病毒蛋白分別和病徵的表現及病原性相關[32, 82, 112]，且都具有基因沉寂抑制子的功用[41, 105, 111] 因而干擾寄主內基因正常的表現所致。由於他們都和寄主的抗性反應具有直接相關性，若能深入研究其中相互作用的機制，並利用其他非表現蛋白的策略，極有潛力發展出有效之抗病植物。

2. RNA 媒介抗性－基因沉寂 (gene silencing)

病原誘導抗性理論中的另一機制爲 RNA 媒介抗性，是指在 RNA 層次上產生分解作用，從而使基因表現被抑制或沉寂，又稱爲 RNA 沉寂 (RNA silencing)。由於植物體內偵測不到轉基因之 mRNA 及其對應蛋白的累積而被認爲抗性之發生在於轉錄後作用的基因沉寂現象 (post-transcriptional gene silencing, PTGS)，一旦該抗性被啓動，則與其序列相同之病毒侵入後便會被分解以達到抗病作用，且提供之抗性具有序列專一性 (sequence-specific) 故對於親緣關係相近的病毒亦具有高專一性的保護效果[9, 29, 42, 106, 107, 114]。Ratcliff 等人[81] 更證明了 PTGS 本身即爲植物抵抗病毒入侵的一種自然防禦反應。利用 PTGS 之原理以基因轉殖方式產生抗病毒之性狀，已成爲近來防治病毒之有效方法。

事實上，此種基因沉寂的現象於植物中是一種調控基因表現與自然防禦的機制，其主要作用機制是當外來或異常的雙股核糖核酸 (dsRNA) 進入細胞內，此 dsRNA 將會被一種名爲 Dicer 的類 RNaseIII 核酸內切酶所辨識，將 dsRNA 切割出 3' 端具有 2 個核苷酸突出的小片段 RNA 分子，大小約爲 21-26 核苷酸 (nucleotide, nt) 即爲小干擾 RNA (small interfering RNA, siRNA)。此分子與其他蛋白結合後形成一複合體，稱爲 RNA-induced silencing complex (RISC)，而 RISC 則利用 siRNA 作爲引導，辨識出與 siRNA 有同源序列的 mRNA 而將 mRNA 分解，此現象亦稱爲 RNA 干擾 (RNA interference)。此外細胞內的 RNA 依賴性 RNA 聚合酶 (RNA-dependent RNA polymerase, RdRP) 可與細胞內的單股 RNA 相結合，合成出雙股 RNA，此雙股 RNA 亦會被 Dicer 所辯識出而被切割，形成更多的 siRNA 分子，而 siRNA 再與 RISC 結合，可分解 (degradation) 更多的 RNA 片段，從而使基因表現被抑制或沉寂。目前已知在植物中引發基因沉寂的路途徑至少可分爲三種：(1) 轉錄時基因沉寂現象 (transcriptional gene silencing, TGS)、(2) 轉錄後基因沉寂現象 (post-transcriptional gene silencing, PTGS) 和 (3) 由 microRNA (miRNA) 所誘導產生之基因沉寂現象[8]。TGS 和 PTGS 現象對植物而言亦爲一種防禦機制，可以抵抗外來核糖核酸片段的侵入，使外來核酸片段不表現。TGS 現象爲將特定或外來核酸序列甲基化而使基因不表現[64]；PTGS 可防止外來的核酸序列在細胞中複製，主要發生在細胞質層次。而 miRNA 主要是參與細胞內調控基因的表現[7]。

雖然雙生病毒屬於 DNA 病毒並在寄主細胞核中進行複製，且複製過程中不會形成dsRNA的中間產物，但仍可誘導基因沉寂機制的產生並成爲基因沉寂機制的攻擊目標[66, 110]。其因素如下：(1) 雙生病毒進行雙方向轉錄 (bi-directional transcription) 時，來自兩相反方向合成之 mRNA 的 3' 端 (如 AC3 及 AV2 的 3' 端) 具有重疊 (overlapping) 的現象，提供了 dsRNA 的來源[21]、(2) 病毒轉錄初期會產生大量之 mRNA，可經由寄主之 RdRP 作用而合成 dsRNA[26]、(3) mRNA 本身所具有的二級結構即可被 Dicer 辨認並進行切割[21]。此外，由於雙生病毒爲 DNA 基因體，故亦有可能經由 TGS 的機制而能有效的降低病毒的轉錄作用。此構想首先有學者利用來自 geminivirus 之啓動子 (promoter) 驅動轉基因的表現，接著再利用 geminivirus 接種帶有此構築之轉基因植物，結果出現了基因沉寂的現象。進一步分析發現其原因是由於啓動子序列高度甲基化 (hypermethylation) 的情形，但此作用只能發生在同源病毒中[91]。此外，自然界中有些植物受雙生病毒感染經過一段時間後所新生的系統葉出現病徵消失的現象，稱之爲恢復型 (recovery) 的寄主植物，經分析後發現於IR可偵測到高度甲基化的現象，證實雙生病毒的 DNA 可成爲 TGS 作用的目標[83]。綜上所述，雙生病毒可誘發並成爲基因沉寂機制的目標，因此我們可以利用基因沉寂的機制作爲防治雙生病毒的策略，並且除了可利用 PTGS 之外，也可經由 TGS 的途徑達到抗病目標。

基因沉寂的策略近來廣爲利用在雙生病毒 (表二) 的抗病研發，並依其使用的基因片段及構築型式不同或是誘發 siRNA 的程度高低，效果亦有所差異。早於基因沉寂機制明確的提出之前，已有研究利用 TGMV 的 AC1 基因互補股核酸 (antisense) 進行轉殖並對病毒

產生延遲發病的情形[27]；隨後轉殖 TYLCV C1 基因互補股核酸 (antisense) 的轉基因菸草則可對病毒產生抗性，且抗性至少可遺傳給二代之子代[12]。Chellappan 等人利用引起樹薯嵌紋病 (cassava mosaic disease, CMD) 的 ACMV 之 AC1 基因進行轉殖，發現除了對 ACMV 具有良好抗性外，還可對抗引起樹薯嵌紋病的其他雙生病毒，包括 *East African cassava mosaic virus* (EACMV) 與 *Sri Lankan cassava mosaic virus* (SLCMV)[20]。此外不論使用病毒正股 (virion sense) 或是互補股核酸的部分 IR 序列加上部分 5' 端 C1 基因的轉基因番茄也發現有良好的抗性，且果實產量不受影響[116]。而利用 ToLCNDV 的 AV2 基因互補股核酸亦有抗病效果，轉基因植物於病毒感染後無病徵出現[68]。近來研究報告指出高程度的基因沉寂可經由雙股髮夾狀 RNA 所觸發[98]，藉此產生高度抗性。因此，近幾年不少研究利用轉殖反向重複序列 (inverted repeats) 的構築來產生雙股髮夾狀 RNA 誘發抗性，亦獲得顯著的效果。例如利用病毒啓動子 (promoter) 序列構築成反向重複序列的構造，進行轉殖，發現可經由 TGS 的途徑達到抗病效果，可將受感染之植物恢復與健康植株相似的樣子[73, 108]。若轉殖 AC1 基因的反向重複序列可獲得對病毒免疫 (immunity) 的抗性植物[37, 109]；而使用鞘蛋白基因的反向重複序列則亦有保護效果，接種病毒 7 周，轉基因植物未有病徵出現[118]。然而目前之研究結果顯示，利用轉基因植物方法發展出之抗性植物雖都具有程度高低不等之抗性效果，但抗性多數只能針對特定病毒而無法具有廣泛性的抗性。其因素可能爲基因沉寂作用具有高度的序列專一性的特點，再者，雙生病毒的 DNA 具高度重組性，致使病毒歧異度增加，新病毒持續發生[15, 90]，相對提高了防治的困難度。因此轉殖高度保留區序列或可利用誘發大量 siRNA 的序列區域並同時針對多個目標基因進行攻擊，或許可提供植物更爲穩定、持久且具廣泛性的抗性。此外，亦有研究指出利用人工合成 miRNA (artificial miRNA) 可產

表二、以基因沉寂法及 DNA 干擾法提供抗性之轉基因策略

Table 2. Transgenic resistance strategies by gene silencing and DNA interference

Gene	Virus species	Transgenic plant	References
Sense			
AC1	ACMV	Cassava	20
CMV CP and C1	ToLCV	*N. tabacum* and tomato	77
Antisense (as)			
as AC1	TGMV	*N. tabacum*	27
as AC1, (AC2, AC3)	TGMV	*N. tabacum*	11
5'-end, as C1	TYLCV	*N. benthamiana*	12
as AC1, AC2, AC3, MP	BGMV	Bean	4
Partial IR+5'-end C1 (sense and as)	TYLCV	Tomato	116
as C1,C2,C3	ACMV	Cassava	117
Partial C1 (sense & as)	CLCuV	*N. tabacum*	5
as C1	ToLCV	Tomato	76
as C1	ToLCV	Tomato	78
as AV2	ToLCNDV	*N. tabacum*	69
Hairpin (inverted repeat)			
IR inverted repeats	MYMV-Vig	Blackgram	73
Intron-hairpin V1V2/C1C2/C2C3	TYLCV complex conserved region	*N. benthamiana* and tomato	1
Hairpin 3'-end AC1	TYLCV	Tomato	37
Hairpin AC1	TGMV	Common bean	13
Hairpin AC1/ AC4	ToLCV	Tomato	80
Hairpin IR	ACMV	Cassava	108
CP inverted repeats	TYLCV	Tomato	118
Hairpin CP or Rep	TYLCSV- [Sic]	*N. benthamiana*	39
Hairpin AC1	ACMV	Cassava	109
DNA interference			
DI DNA (tandem repeat)	ACMV		100
(defective interfering DNA)	BCTV	*N. benthamiana*	101, 35

生對 RNA 病毒具有抗性的轉基因植物[79]，由於此策略乃是以基因沉寂爲基礎並在細胞核內產生作用，因此具有應用於發展雙生病毒抗性的潛力。

3. DNA 干擾

由於雙生病毒爲 DNA 病毒，且於複製過程中會產生干擾缺失性 DNA (defective interfering DNA, DI DNA) 所以有學者藉由轉殖干擾缺失性 DNA 來干擾病毒複製，並可減緩病徵的嚴重性[35, 100, 101] (表二)。此外，此種方式具有病毒專一性[101]，抗病效果和使用的大小相關[35]。

4. 表現非病毒蛋白

除了可藉表現病毒蛋白之外，亦可利用其他非病毒蛋白來產生抗性 (表一)，例如核醣體滅活蛋白 (ribosome-inactive protein, RIP) 爲植物內天然的抗病毒毒素，因此若轉殖核醣體滅活蛋白群中的康乃馨蛋白 (dianthin) 至植物中則有減緩病徵的效果[52]。另外研究指出使用人造鋅指蛋白質 (artificial zinc finger protein, AZP) 可與 Rep 蛋白競爭位於病毒複製起始點的結合位置進而降低甚至抑制病毒的複製[93, 103]。粉蝨體內與傳播病毒相關內共生菌的 GroEL 蛋白亦被轉殖至植物內進行表現，使轉基因植物藉其與病毒鞘蛋白結合的特性以達到減緩病徵的功效[2]。此外利用重組抗體媒介抗性 (recombinant antibody-mediated resistance) 的策略在植物體中表現抗體或抗體的片段來直接對抗抗原的方式，已應用至抵抗 RNA 病毒的感染，近來此策略亦成功應用至抵抗植物 DNA 病毒[85]。首篇研究即是使用與 TYLCV 之 Rep 蛋白可產生辨識作用的單鏈抗體變異區 (single-chain variable fragment, scFv) 基因片段進行轉殖，發現可抑制病毒的複製而達到保護植物的目的[85]。然而不論何種蛋白都需經適當的選擇或修飾之後使用，以避免轉殖至植物後造成不良的影響而失去使用價値。

結　論

現今已發展多種方法來生產抗雙生病毒的轉基因植物 (表一、表二)，雖可獲得程度高低不等的抗性，但多數爲病毒專一性的抗性，僅有非常少數能提供廣泛性的抗性，因此如何尋求對雙生病毒可產生穩定且具廣泛性抗性的策略與方法乃是當前的重要課題。目前的研究成果顯示由核酸層次誘發的抗病轉基因作物，可產生較佳的病毒抗性，例如近來多以反向重複序列的構築誘導高度的基因沉寂作用皆能達到良好抗病的效果。此外經由 TGS 機制所產生的抗性亦具有顯著的效果甚至可達到防治病毒病的功能，因爲 TGS 可經由 siRNA 誘發 DNA 的甲基化，而雙生病毒屬於 DNA 病毒且於寄主細胞核內進行複製，可能因此而能有效率的抑制病毒的複製。因此，若能轉殖高度保留區序列或可利用誘發大量 siRNA 的序列區域並同時針對多個目標基因進行攻擊，如同時採用 IR、Rep、TrAP 等多個區域之高度保留區序列的重組片段，或許可經由不同的抗病機制提供植物更爲穩定、持久且具廣泛性的抗性。另一具有發展雙生病毒抗性潛力的策略是利用人工合成 miRNA (artificial miRNA) 進行轉殖，因其機制是以基因沉寂爲基礎並在雙生病毒進行複製的場所－細胞核內產生作用。此外透過核酸層次的方法不會產生病毒的蛋白產物，其生物安全性較高。若透過表現蛋白產物的策略，有些則會造成轉基因植物外表不正常，因此常使用突變或不完整的蛋白，但須考量使用的蛋白區域及其大小，因爲這些因素皆會影響抗性。除了利用病毒本身的基因蛋白做爲產生抗性的途徑外亦可使用非病毒的物質來干擾病毒的活性。然而，病毒能否於寄主植物中複製且引起病害，決定於病毒和寄主、環境以及傳播媒介相互作用的結果，因此若能充分了解參與病害的機制與要素，便可提供研發抗性轉基因植物的基石，建立正確、穩定、有效率的抗病模式，更有利生產持久且具廣泛性抗性的轉基因植物。

目前越來越多元的策略已逐一被開發，其目標不外乎是想有效的控制病害的發生以減少農業的損失與確保農民收益。本實驗室近年來針對引起台灣番茄重大經濟損失的台灣番茄捲葉病毒 (*Tomato leaf curl Taiwan virus*, ToLCTWV) 進行研究，藉轉殖病毒不同的基因片段分析提供轉基因植物抗性程度的差異[63] 並利用連結數個提供較佳抗性的基因片段研發抗台灣番茄捲葉病毒轉基因植物 (Lin *et al.*, unpublished data)，以期能降低病毒對作物的爲害。除了採用 siRNA 的途徑誘發抗性之外，利用人工合成 miRNA 提供對雙生病毒抗性之可行性目前亦有學者正進行研究分析 (中興大學葉錫東老師實驗室)。此外還有其他新穎的抗病策略或技術目前正持續開發測試中例如以 geminivirus-specific peptide aptamers 的方法產生多重抗性[65] 等。未來也可朝向結合多種策略的方向研究，或許能發展一套具有高效率取具廣泛性的抗病方法。

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引用文獻 (LITERATURE CITED)

1. Abhary, M. K., Anfoka, G. H., Nakhla, M. K., and Maxwell, D. P. 2006. Post-transcriptional gene silencing in controlling viruses of the *Tomato yellow leaf curl virus* complex. Arch. Virol. 151: 2349-2363.
2. Akad, F., Eybishtz, A., Edelbaum, D., Gorovits, R., Dar-Issa, O., Iraki, N., and Czosnek, H. 2007. Making a friend from a foe: expressing a GroEL gene from the whitefly Bemisia tabaci in the phloem of tomato plants confers resistance to *Tomato yellow leaf curl virus*. Arch. Virol. 152: 1323-1339.
3. Antignus, Y., Vunsh, R., Lachman, O., Pearlsman, M., Maslenin, L., Hananya, U., and Rosner, A. 2004. Truncated Rep gene originated from *Tomato yellow leaf curl virus*-Israel [Mild] confers strain-specific resistance in transgenic tomato. Ann. Appl. Biol. 144: 39-44.
4. Aragão, F. J. L., Ribeiro, S. G., Barros, L. M. G., Brasileiro, A. C. M., Maxwell, D. P., Rech, E. L., and Faria, J. C. 1998. Transgenic beans (*Phaseolus vulgaris* L.) engineered to express viral antisense RNAs show delayed and attenuated symptoms to bean golden mosaic geminivirus. Mol. Breed. 4: 491-499.
5. Asad, S., Haris, W. A., Bashir, A., Zafar, Y., Malik, K. A., Malik, N. N., and Lichtenstein, C. P. 2003. Transgenic tobacco expressing geminiviral RNAs is resistant to the serious viral pathogen causing cotton leaf curl disease. Arch. Virol. 148: 2341-2352.
6. Azzam, O., Diaz, O., Beaver, J. S., Gilbertson, R. L., Russell, D. R., and Maxwell, D. P. 1996. Transgenic beans with the bean golden mosaic geminivirus coat protein gene are susceptible to virus infection. Annu. Rep. Bean Improv. Coop. 39: 276-277.
7. Bartel, D. P. 2004. MicroRNAs: genomics, biogenesis, mechanism, and function. Cell 116: 281-297.
8. Baulcombe, D. C. 2004. RNA silencing in plants. Nature 431: 356-363.
9. Baulcombe, D. C., and English, J. J. 1996. Ectopic pairing of homologous DNA and post-transcriptional gene silencing in transgenic plants. Curr. Opin. Biotechnol. 7: 173-180.
10. Behjatnia, S. A. A., Dry, I. B., and Rezaian, M. A. 1998. Identification of the replication-associated protein binding domain within the intergenic region of tomato leaf curl geminivirus. Nucleic Acids Res. 26: 925-931.
11. Bejarano, E. R., and Lichtenstein, C. P. 1994. Expression of TGMV antisense RNA in transgenic tobacco inhibits replication of BCTV but not ACMV geminiviruses. Plant Mol. Biol. 24: 241-248.
12. Bendahmane, M., and Gronenborn, B. 1997. Engineering resistance against tomato yellow leaf curl virus (TYLCV) using antisense RNA. Plant Mol. Biol. 33: 351-357.
13. Bonfim, K., Faria, J. C., Nogueira, E. O., Mendes, E. A., and Aragão, F. J. 2007. RNAi-mediated resistance to *Bean golden mosaic virus* in genetically engineered common bean (*Phaseolus vulgaris*). Mol. Plant-Microbe Interact. 20: 717-726.
14. Briddon, R. W., Mansoor, S., Bedford, I. D., Pinner, M. S., Saunders, K., Stanley, J., Zafar, Y., Malik, K. A., and Markham, P. G. 2001. Identification of DNA components required for induction of cotton leaf curl disease. Virology 285: 234-243.
15. Briddon, R. W., and Stanley, J. 2006. Subviral agents associated with plant single-stranded DNA viruses. Virology 344: 198-210.
16. Briddon, R. W., Watts, J., Markham, P. G., and Stanley, J. 1989. The coat protein of beet curly top virus is essential for infectivity. Virology 172: 628-633.
17. Brunetti, A., Tavazza, R., Noris, E., Lucioli, A., Accotto, G. P., and Tavazza, M. 2001. Transgenically expressed T-Rep of *Tomato yellow leaf curl Sardinia virus* acts as a trans-dominant-negative mutant, inhibiting viral transcription and replication. J. Virol. 75: 10573-10581.
18. Brunetti, A., Tavazza, M., Noris, E., Tavazza, R., Caciagli, P., Ancora, G., Crespi, S., and Accotto, G. P. 1997. High expression of truncated viral Rep protein confers resistance to Tomato yellow leaf curl virus in transgenic tomato plants. Mol. Plant-Microbe Interact. 10: 571-579.
19. Chatterji, A., Beachy, R. N., and Fauquet, C. M. 2001. Expression of the oligomerization domain of the replication-associated protein (Rep) of *Tomato leaf curl New Delhi virus* interferes with DNA accumulation of heterologous geminiviruses. J. Biol. Chem. 276: 25631-25638.
20. Chellappan, P., Masona, M. V., Vanitharani, R., Taylor, N. J., and Fauquet, C. M. 2004a. Broad spectrum resistance to ssDNA viruses associated with transgene-induced gene silencing in cassava. Plant Mol. Biol. 56: 601-611.
21. Chellappan, P., Vanitharani, R., Fauquet, C. M. 2004b. Short interfering RNA accumulation correlates with host recovery in DNA virus-infected hosts, and gene silencing targets specific viral sequences. J. Virol. 78: 7465-7477.

22. Chellappan, P., Vanitharani, R., and Fauquet, C. M. 2005. MicroRNA-binding viral protein interferes with *Arabidopsis* development. Proc. Natl. Acad. Sci. USA 102: 10381-10386.
23. Cooper, B., Lapidot, M., Heick, J. A., Dodds, J. A., and Beachy, R. N. 1995. A defective movement protein of TMV in transgenic plants confer resistance to multiple viruses whereas the functional analog increases susceptibility. Virology 206: 307-313.
24. Cui, X., Li, G., Wang, D., Hu, D., and Zhou, X. 2005. A begomovirus DNA β-encoded protein binds DNA, functions as a suppressor of RNA silencing, and targets the cell nucleus. J. Virol. 79: 10764-10775.
25. Cui, X., Tao, X., Xie, Y., Fauquet, C. M., and Zhou, X. 2004. A DNA β associated with *Tomato yellow leaf curl China virus* is required for symptom induction. J. Virol. 78: 13966-13974.
26. Dalmay, T., Hamilton, A. J., Rudd, S., Angell, S., and Baulcombe, D. C. 2000. An RNA-dependent RNA polymerase gene in *Arabidopsis* is required for posttranscriptional gene silencing mediated by a transgene but not by a virus. Cell 101: 543-553.
27. Day, A. G., Bejarano, E. R., Buck, K. W., Burrell, M., and Lichtenstein, C. P. 1991. Expression of an antisense viral gene in transgenic tobacco confers resistance to the DNA virus tomato golden mosaic virus. Proc. Natl. Acad. Sci. USA 88: 6721-6725.
28. Desbize, C., David, C., Mettouchi, A., Laufs, J., and Gronenborn, R. 1995. Rep protein of tomato yellow leaf curl geminivirus has an ATPase activity required for viral DNA replication. Proc. Natl. Acad. Sci. USA 92: 5640-5644.
29. Dougherty, W. G., Lindbo, J. A., Smith, H. A., Parks, T. D., Swaney, S., and Proebsting, W. M. 1994. RNA-mediated virus resistance in transgenic plants: Exploitation of a cellular pathway possibly involved in RNA degradation. Mol. Plant-Microbe Interact. 7: 544-552.
30. Duan, Y. P., Powell, C. A., Webb, S. E., Purcifull, D. E., and Hiebert, E. 1997. Geminivirus resistance in transgenic tobacco expressing mutated BC1 protein. Mol. Plant-Microbe Interact. 10: 617-623.
31. Fauquet, C. M., Briddon, R. W., Brown, J. K., Moriones, E., Stanley, J., Zerbini, M., and Zhou, X. 2008. Geminivirus strain demarcation and nomenclature. Arch. Virol. 153: 783-821.
32. Fondong, V. N., Reddy, R. V., Chowda, Lu, C., Hankoua, B., and Felton, C. 2007. The consensus N-myristoylation motif of a geminivirus AC4 protein is required for membrane binding and pathogenicity. Mol. Plant-Microbe Interact. 20: 380-391.
33. Fontes, E. P. B., Eagle, P. A., Sipe, P. S., Luckow, V. A., and Hanley-Bowdoin, L. 1994. Interaction between a geminivirus replication protein and origin DNA is essential for viral replication. J. Biol. Chem. 269: 8459-8465.
34. Freitas-Astua, J., Purcifull, D. E., Polston, J. E., and Hiebert, E. 2002. Traditional and transgenic strategies for controlling tomato-infecting begomovirus. Fitopatol. Bras. 27: 437-449.
35. Frischmuth, T., Engel, M., and Jeske, H. 1997. Beet curly top virus DI DNA-mediated resistance is linked to its size. Mol. Breed. 3: 213-217.
36. Frischmuth, S., and Stanley, J. 1998. Recombination between viral DNA and the transgenic coat protein gene of African cassava mosaic geminivirus. J. Gen. Virol. 79: 1265-1271.
37. Fuentes, A., Ramos, P. L., Fiallo, E., Callard, D., Sãnchez, Y., Peral, R., Rodríguez, R., and Pujol, M. 2006. Intron-hairpin RNA derived from replication associated protein C1 gene confers immunity to *Tomato yellow leaf curl virus* infection in transgenic tomato plants. Transgenic Res. 15: 291-304.
38. Ghanim, M., Morin, S., Zeidan, M., and Czosnek, H. 1998. Evidence for transovarial transmission of tomato yellow leaf curl virus by its vector, the whitefly *Bemisia tabaci*. Virology 240: 295-303.
39. Gharsallah-Chouchane, S., Gorsane, F., Nakhla, M. K., Salus, M., Martin, C. T., Maxwell, D. P., Marrakchi, M., and Fakhfakh, H. 2008. Evaluation of two gene-silencing constructs for resistance to tomato yellow leaf curl viruses in *Nicotiana benthamiana* plants. Acta Virol. 52: 143-149.
40. Gilreath, P., Shuler, K., Polston, J. E., Sherwood, T. A., McAvoy, G., Stansly, P. A., and Waldo, E. 2001. *Tomato yellow leaf curl virus* resistant tomato variety trials. Proc. Fla. State Hort. Soc. 114: 190-193.
41. Gopal, P., Kumar, P. P., Sinilal, B., Jose, J., Yadunandam, A., and Usha, R. 2007. Differential roles of C4 and βC1 in mediating suppression of post-transcriptional gene silencing: evidence for transactivation by the C2 of *Bhendi yellow vein mosaic virus*, a monopartite begomovirus. Virus Res. 123: 9-18.
42. Grant, S. R. 1999. Dissecting the mechanisms of posttranscriptional gene silencing: divide and conquer. Cell 96: 303-306.
43. Grumet, R. 1995. Genetic engineering for crop virus resistance. Hortscience 30: 449-456.
44. Guo, W., Jiang, T., Zhang, X., Li, G., and Zhou, X. 2008. Molecular variation of satellite DNA beta molecules associated with *Malvastrum yellow vein virus* and their role in pathogenicity. Appl. Environ. Microbiol. 74: 1909-1913.

45. Hao, L., Wang, H., Sunter, G., and Bisaro, D. M. 2003. Geminivirus AL2 and L2 proteins interact with and inactivate SNF1 kinase. Plant Cell 15: 1034-1048.
46. Hanley-Bowdoin, L., Settlage, S. B., Orozco, B. M., Nagar, S., and Robertson, D. 1999. Geminiviruses: models for plant DNA replication, transcription, and cell cycle regulation. Crit. Rev. Plant Sci. 18: 71-106.
47. Hanley-Bowdoin, L., Settlage, S. B., and Robertson, D. 2004. Reprogramming plant gene expression: a prerequisite to geminivirus DNA replication. Mol. Plant Pathol. 5: 149-156.
48. Hilje, L., Costa, H. S., and Stansly, P. A. 2001. Cultural practices for managing *Bemisia tabaci* and associated viral diseases. Crop Protect. 20: 801-812.
49. Hofer, P., Bedford, I. D., Markham, P. G., Jeske, H., and Frischmuth, T. 1997. Coat protein gene replacement results in whitefly transmission of an insect non-transmissible geminivirus isolate. Virology 236: 288-295.
50. Hong, Y., and Stanley, J. 1995. Regulation of African cassava mosaic virus complementary-sense gene expression by N-terminal sequences of the replication-associated protein AC1. J. Gen. Virol. 76: 2415-2422.
51. Hong, Y., and Stanley, J. 1996. Virus resistance in *Nicotiana benthamiana* conferred by African cassava mosaic virus replication-associated protein (AC1) transgene. Mol. Plant-Microbe Interact. 9: 219-225.
52. Hong, Y., Saunders, K., Hartley, M. R., and Stanley, J. 1996. Resistance to geminivirus infection by virus-induced expression of dianthin in transgenic plants. Virology 220: 119-127.
53. Hou, Y. M., Sanders, R., Ursin, V. M., and Gilbertson, R. L. 2000. Transgenic plants expressing geminivirus movement proteins: abnormal phenotypes and delayed infection by *Tomato mottle virus* in transgenic tomatoes expressing the *Bean dwarf mosaic virus* BV1 or BC1 proteins. Mol. Plant-Microbe Interact. 13: 297-308.
54. Ingham, D. J., Pascal, E., and Lazarowitz, S. G. 1995. Both bipartite geminivirus movement proteins define viral host range, but only BL1 determines viral pathogenicity. Virology 207: 191-204.
55. Jose, J., and Usha, R. 2003. Bhendi yellow vein mosaic disease in India is caused by association of a DNA- satellite with a *Begomovirus*. Virology 305: 310-317.
56. Kon, T., Hidayat, S. H., Hase, S., Takahashi, H., and Ikegami, M. 2006. The natural occurrence of two distinct begomoviruses associated with DNA β and a recombinant DNA in a tomato plant from Indonesia. Phytopathology 96: 517-525.
57. Kon, T., Sharma, P., and Ikegami, M. 2007. Suppressor of RNA silencing encoded by the monopartite tomato leaf curl Java begomovirus. Arch. Virol. 152: 1273-1282.
58. Krake, L. R., Rezaian, A. M., and Dry, I. B. 1998. Expression of tomato leaf curl C4 gene produces virus-like symptoms in transgenic plants. Mol. Plant-Microbe Interact. 11: 413-417.
59. Kunik, T., Salomon, R., Zamir, D., Navot, N., Zeidan, M., Michelson, I., Gafni, Y., and Czosnek, H. 1994. Transgenic tomato plants expressing the tomato yellow leaf curl virus capsid protein are resistant to the virus. Bio/Technology 12: 500-504.
60. Lapidot, M., and Friedman, M. 2002. Breeding for resistance to whitefly-transmitted geminiviruses. Ann. Appl. Biol. 140: 109-127.
61. Lapidot, M., Friedmann, M., Pilowsky, M., Ben-Joseph, R., and Cohen, S. 2001. The effect of host resistance on *Tomato yellow leaf curl virus* (TYLCV) on virus acquisition and transmission by its whitefly vector. Phytopathology 91: 1209-1213.
62. Laufs, J., Traut, W., Heyraud, F., Matzeit, V., Rogers, S., Schell, J., and Gronenborn, B. 1995. *In vitro* cleavage and joining at the viral origin of replication by the replicaion initiator protein of tomato yellow leaf curl virus. Proc. Natl. Acad. Sci. USA 92: 3879-3883.
63. Lin, C.-Y., Tsai, W.-S., Green, S. K., and Jan, F.-J. 2007. Evaluation of the global genome of *Tomato leaf curl virus* for controlling geminiviruses via gene silencing. The American Phytopathological Society/ the Society of Nematologists Joint Meeting, San Diego, California, USA. Phytopathology 97: S22.
64. Lippman, Z., and Martienssen, R. 2004. The role of RNA interference in heterochromatic silencing. Nature 431: 364-370.
65. Lopez-Ochoa, L., and Hanley-Bowdoin, L. 2007. Use of peptide aptamers for broad-based geminivirus disease resistance. Conference abstract, American Society of Plant Biologists Plant Biology and Botany Joint Congress, Chicago, USA. http://abstracts.aspb.org/pb2007/public/M04/M04003.html
66. Lucioli, A., Noris, E., Brunetti, A., Tavazza, R., Ruzza, V., Castillo, A. G., Bejarano, E. R., Accotto, G. P., and Tavazza, M. 2003. *Tomato yellow leaf curl Sardinia virus* rep-derived resistance to homologous and heterologous geminiviruses occurs by different mechanisms and is overcome if virus-mediated transgene silencing is activated. J. Virol. 77: 6785-6798.
67. Matsuda, N., Sharma, P., Bajet, N. B., and Ikegami, M. 2008. Molecular characterization of a new strain of *Tomato leaf curl Philippines virus* and its associated satellite DNA beta molecule: further evidence for

natural recombination amongst begomoviruses. Arch. Virol. 153: 961-967.

68. Morin, S., Ghanim, M., Sobol, I., and Czosnek, H. 2000. The GroEL protein of the whitefly *Bemisia tabaci* interacts with the coat protein of transmissible and non-transmissible begomoviruses in the yeast two-hybrid system. Virology 276: 404-416.
69. Mubin, M., Mansoor, S., Hussain, M., and Zafar, Y. 2007. Silencing of the AV2 gene by antisense RNA protects transgenic plants against a bipartite begomovirus. Virol. J. 4: 10.
70. Noris, E., Accotto, G. P., Tavazza, R., Brunetti, A., Crespi, S., and Tavazza, M. 1996. Resistance to tomato yellow leaf curl geminivirus in *Nicotiana benthamiana* plants transformed with a truncated viral C1 gene. Virology 224: 130-138.
71. Noris, E., Vaira, A. M., Caciagli, P., Masenga, V., Gronenborn, B., Accotto, G. P. 1998. Amino acids in the capsid protein of tomato yellow leaf curl virus that are crucial for systemic infection, particle formation, and insect transmission. J. Virol. 72: 10050-10057.
72. Pascal, E., Goodlove, P. E., Wu, L. C., and Lazarowitz, S. G. 1993. Transgenic tobacco planta expressing the geminivirus BL1 protein exhibit symptoms of viral disease. Plant Cell 5: 795-807.
73. Pooggin, M., Shivaprasad, P. V., Veluthambi, K., and Hohn, T. 2003. RNAi targeting of DNA virus in plants. Nat. Biotechnol. 21: 131-132.
74. Pooma, W., Gillette, W. K., Jeffrey, J. L., and Petty, I. T. 1996. Host and viral factors determine the dispensability of coat protein for bipartite geminivirus systemic movement. Virology 218: 264-268.
75. Powell-Abel, P., Nelson, R. S., De, B., Hoffmann, N., Rogers, S. G., Fraley, R. T., and Beachy, R. N. 1986. Delay of disease development in transgenic plants that express the tobacco mosaic virus coat protein gene. Science 232: 738-743.
76. Praveen, S., Kushwaha, C. M., Mishra, A. K., Singh, V., Jain, R. K., and Varma, A. 2005. Engineering tomato for resistance to tomato leaf curl disease using viral *rep* gene sequences. Plant Cell Tissue Organ Cult. 83: 311-318.
77. Praveen, S., Mishra, A. K., and Antony, G. 2006. Viral suppression in transgenic plants expression chimeric transgene from *Tomato leaf curl virus* and *Cucumber mosaic virus*. Plant Cell Tissue Organ Cult. 84: 47-53.
78. Praveen, S., Mishra, A. K., and Dasgupta, A. 2005. Antisense suppression of replicase gene expression recovers tomato plants from leaf curl infection. Plant Sci. 168: 1011-1014.
79. Qu, J., Ye, J., Fang, R. 2007. Artificial MicroRNA-mediated virus resistance in plants. J. Virol. 81: 6690-6699.
80. Ramesh, S. V., Mishra, A. K., and Praveen, S. 2007. Hairpin RNA-mediated strategies for silencing of *Tomato leaf curl virus* AC1 and AC4 genes for effective resistance in plants. Oligonucleotides 17: 251-257.
81. Ratcliff, F. G., MacFariance, S. A., and Baulcome, D. C. 1999. Gene silencing without DNA: RNA-mediated cross-protection between virus. Plant Cell 11: 1207-1215.
82. Rigden, J. E., Krake, L. R., Rezaian, M. A., Dry, I. B. 1994. ORF C4 of tomato leaf curl geminivirus is a determinant of symptom severity. Virology 204: 847-850.
83. Rodriguez-Negrete, E. A., Carrillo-Tripp, J., and Rivera-Bustamante, R. F. 2009. RNA silencing against geminivirus: complementary action of post-transcriptional gene silencing and transcriptional gene silencing in host recovery. J. Virol. 83: 1332-1340.
84. Rojas, M. R., Jiang, H., Salati, R., Xoconostle-Cãzares, B., Sudarshana, M. R., Lucas, W. J., and Gilbertson, R. L. 2001. Functional analysis of proteins involved in movement of the monopartite begomovirus, *Tomato yellow leaf curl virus*. Virology. 291: 110-125.
85. Safarnejad, M. R., Fischer, R., and Commandeur, U. 2009. Recombinant-antibody-mediated resistance against *Tomato yellow leaf curl virus* in *Nicotiana benthamiana*. Arch. Virol. 154: 457-467.
86. Sanderfoot, A. A., Ingham, D. J., and Lazarowitz, S. G. 1996. A viral movement protein as a nuclear shuttle. Plant Physiol. 110: 23-33.
87. Sanford, J. C., and Johnston, S. A. 1985. The concept of parasite-derived resistance-deriving resistance genes from the parasite's own genome. J. Theor. Biol. 113: 395-405.
88. Sangare, A., Deng, D., Fauquet, C. M., and Beachy, R. N. 1999. Resistance to *African cassava mosaic virus* conferred by mutant of the putative NTP-binding domain of the Rep gene (AC1) in *Nicotiana benthamiana*. Mol. Breed. 5: 95-102.
89. Saunders, K., Bedford, I. D., Briddon, R. W., Markham, P. G., Wong, S. M., and Stanley, J. 2000. A unique virus complex causes *Ageratum* yellow vein disease. Proc. Natl. Acad. Sci. USA 97: 6890-6895.
90. Seal, S. E., van den Bosch, F., and Jeger, M. J. 2006. Factors influencing begomovirus evolution and their increasing global significance: implications for sustainable control. Crit. Rev. Plant Sci. 25: 23-46.
91. Seemanpillai, M., Dry, I., Randles, J., and Rezaian, A. 2003. Transcriptional silencing of geminiviral promoter-driven transgenes following homologous

virus infection. Mol. Plant-Microbe Interact. 16: 429-438.

92. Selth, L. A., Dogra, S. C., Rasheed, M. S., Healy, H., Randles, J. W., and Rezaian, M. A. 2005. A NAC domain protein interacts with *Tomato leaf curl virus* replication accessory protein and enhances viral replication. Plant Cell 17: 311-325.
93. Sera, T. 2005. Inhibition of virus DNA replication by artificial zinc finger proteins. J. Virol. 79: 2614-2619.
94. Shepherd, D. N., Mangwende, T., Martin, D. P., Bezuidenhout, M., Thomson, J. A., and Rybicki, E. P. 2007. Inhibition of *Maize streak virus* (MSV) replication by transient and transgenic expression of MSV replication-associated protein mutants. J. Gen. Virol. 88: 325-336.
95. Shepherd, D. N., Mangwende, T., Martin, D. P., Bezuidenhout, M., Kloppers, F. J., Carolissen, C. H., Monjane, A. L., Rybicki, E. P., and Thomson, J. A. 2007. *Maize streak virus*-resistant transgenic maize: a first for Africa. Plant Biotechnol. J. 5: 759-767.
96. Shivaprasad, P., Thillaichidambaram, P., Balaji, V., and Veluthambi, K. 2006. Expression of full-length and truncated Rep genes from *Mungbean yellow mosaic virus*-Vigna inhibits viral replication in transgenic tobacco. Virus Genes 33: 365-374.
97. Sinisterra, X. H., Polston, N. J. E., Abouzid, A. M., Hiebert, E. 1999. Tobacco plants transformed with a modified coat protein of tomato mottle begomovirus show resistance to virus infection. Phytopathology 89: 701-706.
98. Smith, N. A., Singh, S. P., Wang, M. B., Stoutjesdijk, P. A., Green, A. G., and Waterhouse, P. M. 2000. Total silencing by intron-spliced hairpin RNAs. Nature 407: 319-320.
99. Stanley, J., Bisaro, D. M., Briddon, R. W., Brown, J. K., Fauquet, C. M., Harrison, B. D., Rybicki, E. P., and Stenger, D. C. 2005. Geminiviridae. In Virus Taxonomy. Eighth Report of the International Committee on Taxonomy of Viruses, pp. 301-326. Edited by C. M. Fauquet, M. A. Mayo, J. Maniloff, U. Desselberger & L. A. Ball. London: Elsevier/Academic Press.
100. Stanley, J., Frischmuth, T., and Ellwood, S. 1990. Defective viral DNA ameliorates symptoms of geminivirus infection in transgenic plants. Proc. Natl. Acad. Sci. USA 87: 6291-6295.
101. Stenger, D. C. 1994. Strain-specific mobilization and amplification of a transgenic defective-interfering DNA of the geminivirus beet curly top virus. Virology 203: 397-402.
102. Sunter, G., Sunter, J., and Bisaro, D. M. 2001. Plants expressing *Tomato golden mosaic virus* AL2 or beet curly top virus L2 transgenes show enhanced susceptibility to infection by DNA and RNA viruses. Virology 285: 59-70.
103. Takenaka, G., Koshino-Kimura, Y., Aoyama, Y., and Sera, T. 2007. Inhibition of *Tomato yellow leaf curl virus* replication by artificial zinc-finger proteins. Nucleic Acids Symp. Ser. 51: 429-430.
104. Tripathi, S., and Varma, A. 2002. Eco-friendly management of leaf curl disease of tomato. Indian Phytopath. 55: 473-478.
105. Trinks, D., Rajeswaran, R., Shivaprasad, P. V., Akbergenov, R., Oakeley, E. J., Veluthambi, K., Hohn, T., and Pooggin, M. 2005. Suppression of RNAsilencing by a geminivirus nuclear protein, AC2, correlates with transactivation of host genes. J. Virol. 79: 2517-2527.
106. Wassenegger, M., and Pelissier, T. 1998. A model for RNA-mediated gene silencing in higher plants. Plant Mol. Biol. 37: 349-362.
107. van den Boogaart, T., Lomonossoff, G. P., and Davies, J. W. 1998. Can we explain RNA-mediated virus resistance by homology-dependent gene silencing? Mol. Plant-Microbe Interact. 11: 717-723.
108. Vanderschuren, H., Akbergenov, R., Pooggin, M. M., Hohn, T., Gruissem, W., and Zhang, P. 2007. Transgenic cassava resistance to *African cassava mosaic virus* is enhanced by viral DNA-A bidirectional promoter-derived siRNAs. Plant Mol. Biol. 64: 549-557.
109. Vanderschuren, H., Alder, A., Zhang, P., and Gruissem, W. 2009. Dose-dependent RNAi-mediated geminivirus resistance in the tropical root crop cassava. Plant Mol. Biol. 70: 265-272.
110. Vanitharani, R., Chellappan, P., and Fauquet, C. 2003. Short interfering RNA mediated interference of gene expression and viral DNA accumulation in cultured plant cells. Proc. Natl. Acad. Sci. USA 100: 9632-9636.
111. Vanitharani, R., Chellappan, P., Pita, J. S., and Fauquet, C. 2004. Differential roles of AC2 and AC4 of cassava geminiviruses in mediating synergism and suppression of posttranscriptional gene silencing. J. Virol. 78: 9487-9498.
112. van Wezel, R., Liu, H., Tien, P., Stanley, J., and Hong, Y. 2001. Gene C2 of the monopartite geminivirus *Tomato yellow leaf curl virus*-China encodes a pathogenicity determinant that is localized in the nucleus. Mol. Plant-Microbe Interact. 14: 1125-1128.
113. Varma, A., and Malathi, V. G. 2003. Emerging geminivirus problems: a serious threat to crop production. Ann. Appl. Biol. 142: 145-164.

114. Vaucheret, H., Christophe, B., Elmayan, T., Feuerbach, F., Godon, C., Morel, J. B., Mourrain, P., Palauqui, J. C., and Vernhettes, S. 1998. Transgene-induced gene silencing in plants. Plant J. 16: 651-659.
115. von Arnim, A., and Stanley, J. 1992. Inhibition of *African cassava mosaic virus* systemic infection by a movement protein from the related geminivirus *Tomato golden mosaic virus*. Virology 187: 555-564.
116. Yang, Y., Sherwood, T. A., Patte, C. P., Hiebert, E., and Polston, J. E. 2004. Use of *Tomato yellow leaf curl virus* (TYLCV) Rep gene sequences to engineer TYLCV resistance in tomato. Phytopathology 94: 490-496.
117. Zhang, P., Vanderschuren, H., Futterer, J., and Gruissem, W. 2005. Resistance to cassava mosaic disease in transgenic cassava expressing antisense RNAs targeting virus replication genes. Plant Biotechnol. J. 3: 385-397.
118. Zrachya, A., Kumar, P. P., Ramakrishnan, U., Levy, Y., Loyter, A., Arazi, T., Lapidot, M., and Gafni, Y. 2007. Production of siRNA targeted against TYLCV coat protein transcripts leads to silencing of its expression and resistance to the virus. Transgenic Res. 16: 385-398.

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ABSTRACT

Lin, C.-Y.[1], Tsai, W.-S.[2], Ku, H.-M.[3,4], and Jan, F.-J.[1,4]. 2009. Transgenic strategies for developing transgenic plants with geminivirus resistance. Plant Pathol. Bull. 18: 185-200. ([1] Department of Plant Pathology, National Chung Hsing University, Taichung, Taiwan; [2] Asian Vegetable Research and Development Center-the World Vegetable Center, Tainan, Taiwan; [3] Department of Plant Pathology, National Chung Hsing University, Taichung, Taiwan; [4] Corresponding author, E-mail: fjjan@nchu.edu.tw or hmku@nchu.edu.tw, Fax: +886-4-2285-4145)

Geminiviruses are a serious threat to agriculture practice worldwide. The diseases cause high yield losses, especially in the economical crops, generating serious reduction of farmers' income and food supply in general. In order to solve these economic and social impacts, various strategies have been developed for virus control. Due to their single-stranded DNA genomes and high diversity caused by frequently recombination intra- and inter-virus species, many traditional approaches have limited efficiency for virus control. Therefore, transgenic approach based on genetic engineering technology provides other efficient strategies. This article reviewed the recent studies related to transgenic resistance of geminivirus by different strategies, such as expression of viral or non-viral proteins, gene silencing and DNA interference. The implementation of these strategies will help us to comprehend the resistant model and also contributed to develop the transgenic plants with broad-spectrum and durable resistance.

Key words: geminivirus, transgenic plant, viral resistance